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**Investigating the Interactions between Social Behaviour and Habituation to a Novel Environment in Sticklebacks (Gasterosteus aculeatus)**

# Investigating the Interactions between Social Behaviour and Habituation to a Novel Environment in Sticklebacks (*Gasterosteus aculeatus*)

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# Abstract

This thesis aims to investigate the interactions between the collective behaviour of sticklebacks (*Gasterosteus aculeatus*) and the rate to which they habituate to a novel environment. The first data chapter is a group-based study, which investigates the rate at which groups of varying cohesiveness habituate to a novel environment. Groups of eight individuals were introduced in to a tank containing two refuges and an open area, which was assumed to carry a higher perceived degree of predation risk than the refuges. There was found to be a positive correlation between groups' cohesiveness and the degree to which they habituated to the novel environment, suggesting that behaving collectively may convey a significant fitness advantage through facilitating faster environmental habituation.

The second data chapter used an individual-based approach to investigate the effect of individual personality traits (in terms of boldness and sociability) on the habituation rate of individuals over consecutive days. Individuals were introduced in to a novel environment each day for three consecutive days. Their sociability was quantified by the time that they chose to spend in close proximity to a visible shoal of conspecifics, and the rate at which they habituated to the environment over the course of the tree days was measured. This study found no significant correlation between an individual's sociability and the degree to which they habituated to the environment. However, this study did provide evidence for boldness being a personality trait in sticklebacks.

There was evidence across both of the studies that suggested that several individuals did not habituate to their environments to a significant degree over the course of the trials. This may be due to some limitations in the methods used in these two studies. Recommendations for further study to prevent a desensitization effect from occurring (as was suspected in this study) have been discussed.

# Dedication and Acknowledgements

*I would like to particularly thank my supervisor, Dr. Christos Ioannou, for his support, guidance and patience throughout this research project, and for keeping me on track. Thank you also to everybody from the University of Bristol involved in the training courses, administration and delivery of the Masters by Research course.*

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*A final thank you to Tom Clarkson and everybody at Clarkson & Woods Ecological Consultants Ltd, who have been incredibly supportive in the past six months, and for allowing me time to write up this thesis.*

# Authors Declaration

*I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.*

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## Introduction

Living in groups is a hugely widespread trait, which is found across many animal taxa. Wilson (1975) defined a group as 'any set of organisms, belonging to the same species, that remain together for a period of time interacting with one another to a distinctly greater degree than with other conspecifics'. Groups can be largely stable over time – such as primate groups, where the composition of groups can change very little over an individual's lifetime (Krause *et al*. 2014) – or can be volatile and change in size and composition from minute to minute. For example, shoaling fish have been found to alter their group size in response to dynamic environmental contexts, including the risk of predation and availability of food (Hoare *et al*. 2004).

In order for natural selection to favour the evolution of social behaviour, the net fitness benefits available to an individual remaining in a group must outweigh the fitness benefits of an individual living solitarily. There are many advantages and disadvantages to group living that are well documented in the literature. An advantage of group living is that members of a group may benefit from an increased ability to find resources such as food patches (Pitcher *et al*. 1982). This is particularly beneficial when food exists in bountiful, but scarce patches within an environment, which individuals may struggle to locate independently. However, these potential benefits are opposed by an increased competition for resources when in a group, as a food patch may not hold enough food for the entire group. This can force a large group to increase their foraging effort, spending more energy finding food patches than smaller groups or solitary individuals (Janson 1988).

One of the key drivers of group behaviour is that individuals in groups experience a reduced risk of predation (Lima & Dill 1990) relative to solitary individuals in a similar environment. This is achieved by a combination of 'dilution', 'detection', and 'confusion' effects. Dilution effects refer to the sharing of predation risk across members of a group, statistically reducing the risk of predation for each individual in the group (Foster & Treherne 1981; Morgan & Godin 1985).

Detection effects, also known as the 'many-eyes' effect, states that a group has a higher likelihood of detecting predators than a solitary individual, due to a higher number of individuals that can be vigilant for predators (Elgar & Catterall 1981). This higher shared vigilance allows grouped individuals to reduce their own vigilance effort (Quenette & Gerard 1992; Roberts 1996; Childress & Lung 2003), which in turn enables individuals to spend more time exhibiting other behaviours, such as foraging (Lian *et al.* 2007; Rieucau & Martin 2008), without suffering from an increased predation risk as a result.

The confusion effect refers to the reduction in success rate of predator attacks that can be attributed to the difficulty of tracking and attacking one target when many targets are available simultaneously (Ruxton *et al.* 2007). The accuracy of predator attacks tends to reduce with larger prey group sizes, as high numbers of available targets induce poor neural mapping of prey locations by predators (Ioannou *et al.* 2008). This reduces the predation risk experienced by members of a large group.

### The Mechanisms of Group Behaviour

Many species across multiple taxa show a tendency to form social groups (Shaw 1978), suggesting that this trait has evolved independently many times, albeit to varying extents, across the animal kingdom. It is clear that behaving socially has a strong evolutionary function; by reducing the risk of predation of individuals, grouping can provide strong potential fitness benefits. Although the effects that provide this evolutionary function are relatively complex, the mechanisms that underpin collective behaviour are fairly simple, and occur largely at the local level.

Individual-based computer models have demonstrated that realistic collective group movement can be reproduced when a few simple behavioural rules dictate the spatial positioning of an individual within a group (Couzin *et al.* 2002). Firstly, individuals should avoid collisions with other group members by maintaining a small zone of repulsion between themselves and neighbours.

Secondly, individuals should be attracted towards other individuals in order to remain part of the group, and should also tend to align their direction of movement with their neighbours (Couzin *et al.* 2002). When individuals follow these simple rules at the local level, highly complex collective behaviour emerges across the entire group, such as schooling and shoaling. This phenomenon is known as self-organization, whereby 'patterns at the global level emerge solely from interactions among lower-level components' (Camazine *et al.* 2003).

Groups of fish tend to spend the majority of time in one of three stable collective states – as a polarized school, a torus, or a shoal (Pitcher & Parrish 1993). Shoals are discrete, cohesive groups, but are relatively disordered at the individual level, as individuals are not highly aligned (or, polarized) in their direction of movement. Individuals in a school or a torus align their direction of movement with their neighbours and are therefore polarized; however, groups in a torus formation rotate about an empty core (thus displaying little to no net movement across a space), whereas individuals in a school do show net movement across space. Both theoretical models (Couzin *et al.* 2002) and studies using real subjects (golden shiners, *Notemigonus crysoleucas*) (Tunstrøm *et al.* 2013) have demonstrated that intermediate states between these three formations are relatively unstable, and as a result, groups tend to spend the majority of time in one of these three stable states.

### Personal and Social information

In order to follow these simple local rules, an individual must constantly monitor and process information regarding the behaviour and movements of its neighbours. At any given time, an individual within a group is obtaining information – both privately from sensory information about its environment, and socially from other group members. Social information can be acquired in two ways: through signals, whereby information is intentionally transferred between individuals within a group (i.e. an alarm call, alerting conspecifics to a potential attack); or through unintentional cues, whereby the behaviour of others can unintentionally transfer information to an individual (Dall *et al.* 2005). Social cues that may be useful to an individual include: the flight response

of other conspecifics, revealing the location of a predator attack; feeding by conspecifics, revealing the location of a food patch (Galef and Giraldeau 2001); and mating behaviours, indicating the presence of a potential mate for the focal individual (Nordell & Valone 1998).

Social cues also play a critical role in social learning, particularly with regard to learning where, when, and what, to forage (Galef and Giraldeau 2001). This is demonstrated in studies such as Fryday & Greig-Smith (1994), whereby red-winged blackbirds (*Agelaius phoeniceus*) preferentially fed on the same coloured food as they witnessed their conspecifics feeding on. In this example, observing conspecifics feeding on food of a particular colour acts as a behavioural cue, unintentionally transmitting information from the feeding individual to the observing individual, regarding the palatability of food of a particular colour. Social cues are also used by individuals to acquire information about predators, and to learn certain antipredator behaviours (Griffin 2004). For example, juvenile Belding's ground squirrels (*Spermophilus beldingi*) observing adults respond to an alarm call affects the rate at which they themselves develop an antipredator response to an alarm call (Mateo & Holmes 1997).

Being in a coordinated and cohesive group allows each individual to benefit from social cues and signals transmitted by other members of the group (Couzin and Krause 2003; Ward *et al*. 2008). This transfer of social signals and cues between grouped individuals can allow uninformed members of groups to make correct decisions (the decision that maximises their fitness) approximately as often as well-informed individuals of the group (Magurran & Higham 1988; King & Cowlishaw 2007). For example, often, only a few individuals in a shoal initially detect a predator, but this information is transferred to other uninformed members of the group by the utilisation of social cues. Krause (1993) demonstrated that this kind of information transfer occurs using mixed shoals of chub (*Leuciscus cephalus*) and three-spined sticklebacks (*Gasterosteus aculeatus*). After introducing a shoal to an alarm substance that chub, but not sticklebacks, are sensitive to, Krause demonstrated that sticklebacks respond to the predator avoidance behaviour of the chub by also displaying predator avoidance

behaviour. This suggests that the sticklebacks used social cues from their neighbours (the fright response of the chub to the alarm substance) to gather information about their environment, and used this information to inform their decision to also exhibit antipredator behaviour. Furthermore, when grouped with chub that were habituated to the alarm substance (and therefore did not produce a fright response to it), the sticklebacks also produced no response, confirming that it was the social cue produced by the chub's fright response to the substance that was guiding the stickleback's behaviour.

When observing a highly coordinated group of animals (e.g. a starling murmuration or a school of fish) react to a predator attack, it is clear that social cues, in the form of predator avoidance behaviour, are transferred rapidly between individuals, spreading in waves that propagate incredibly fast across the entire group (Hemelrijk *et al.* 2015). Sometimes, the speed at which predator avoidance behaviour spreads can be faster than the speed at which the predator attacks a group (Treherne & Foster 1981; Marras *et al.* 2012), resulting in a reduced predator success rate (Procaccini *et al.* 2011). This phenomenon is known as the Trafalgar Effect.

Information can be transferred more efficiently within a highly polarised group (Day *et al*. 2001), as any changes in the orientation of neighbours (for example, in response to a predator) can be detected more easily when all individuals have similar orientations. Therefore, the torus and school formations both allow individuals to receive the benefits associated with a high degree of group alignment. However, if a group is cohesive, but not polarised (i.e. more of a swarm/shoal formation, where group members are not aligned in their orientation), cues such as predator avoidance manoeuvres are transmitted less efficiently (Couzin *et al.* 2002).

### Habituation

When an individual finds itself in a novel or changing environment, available information about the environment (i.e. the presence or location of predators) is minimal. Welker and Welker (1958) demonstrated that fish introduced to

novelty initially respond by retreating, and suspending their activity (i.e. freezing). This initial behavioural response is known generally as a 'fright response'. These behavioural responses to novelty suggest that individuals may associate novel environments with a high-perceived risk of predation.

Miller and Gerlai (2012) found that groups of zebrafish (*Danio rerio*) introduced to a novel environment initially tended to behave very collectively, forming polarized schools, but reduced their degree of collectiveness (tending to more often exist in shoals, rather than schools) following several exposures to the environment, as well as over the duration of a single exposure. This initial peak in collective behaviour further suggests that individuals associate novel environments with a high predation risk, and respond to this by exhibiting antipredator behaviour - forming a polarized school over a loose shoal in these high-risk situations. Schools may reduce each individual's risk of predation, relative to a loose shoal, by confusing approaching predators to a greater degree (Bode *et al.* 2010; Ioannou *et al.* 2012), and by increasing the ease of transmission of behavioural cues (such as sudden changes in direction by individuals in response to the location of a predator) between conspecifics, potentially resulting in an increased ability to avoid predators. For these reasons, schooling is considered an antipredator behaviour (Magurran 1990).

However, as groups do not indefinitely exist in a highly polarised school, it is likely that there are costs associated with being a member of a school. Schooling may simply be more energetically costly than shoaling. For example, studies have found that schools tend to travel faster than less organised shoals (Parrish *et al.* 2002; Miller and Gerlai 2012). However, it is worth noting that this effect is likely a result of shoal members having unpolarized orientations and thus travelling across space slowly, rather than polarized groups actively favouring faster travel speeds.

There may also be other costs associated with schooling, such as a reduced potential for individuals to forage when part of a coordinated school. This could occur as a result of intense competition between group members for food

resources, particularly as the field of view of an individual within a polarised group is likely to overlap with many of its neighbours' fields of view (Eggers 1976). Given that food patches are often only available for a limited period of time, it may be more beneficial under certain circumstances to independently forage, because a food patch may be depleted by the time the entire group (and thus some individuals within a group) reaches the food patch. Therefore, an individual may obtain a greater energetic benefit from a food source if it is discovered independently from a group (Dechaume-Moncharmont *et al.* 2005). This indicates that there is a trade-off associated with being a member of a polarised group. Group members may reduce their risk of predation, but potentially at an energetic cost, through the loss of foraging opportunities.

As a result, it is optimal for individuals to only form polarised schools when the perceived risk of predation is high. When the perceived predation risk is low (for example, following habituation to a novel environment), the costs of schooling may outweigh the requirement for antipredator behaviour; therefore individuals may obtain the greatest benefit to fitness by reducing their collectiveness, and exploring or foraging alone, or in a less polarised group. The process of becoming accustomed to a novel environment, and the equalisation of the perceived risk and actual risk of the environment is referred to in this study, and others (Miller and Gerlai 2012), as environmental habituation. Note that the definition used in this study slightly differs to the classical definition of habituation, whereby the response of an individual to a non-threatening stimulus reduces over repeated exposures. In this study, environmental habituation is essentially the reduction of antipredator behaviour as an individual becomes accustomed to a novel environment over time, as there is no actual risk of predation in the experimental setups.

Environmental habituation is expected to occur once an individual has made an accurate assessment of the predation risk of a particular environment. If an individual's perceived risk of predation is higher than the actual predation risk of a given environment for a prolonged period, individuals may waste time and energy investing in antipredator behaviours (such as using refuges or schooling

with conspecifics), when they could be exhibiting other behaviours that benefit fitness, such as foraging. Thus, habituating to a novel environment as quickly as possible may have an important potential benefit to an individual's fitness. This is demonstrated in Rodriguez-Prieto *et al.* (2010a), where Iberian wall lizards (*Podarcis hispanica*) that habituated faster to a frequently encountered low-risk predator had better body condition than individuals who habituated to the predator to a lesser extent.

If the sharing of social information between group members can increase an individual's rate of environmental habituation, this may provide individuals who behave collectively a fitness benefit by optimizing their energy usage. If this were the case, this would suggest that information transfer may have an important role in increasing the fitness of collective individuals, and thus may also be a driver of the evolution of social behaviour.

This thesis aims to investigate the interactions between collective behaviour and environmental habituation. The first data chapter is a group-based study which investigates the rate at which groups of varying collectiveness habituate to a novel environment, in order to assess whether more efficient information transfer between more collective groups can facilitate a faster rate of environmental habituation. The second data chapter uses an individual-based approach to investigate the effect of individual personality traits (in terms of boldness and sociability) on the habituation rate of individuals over consecutive days.

Both of the data chapters within this thesis use a group's cohesiveness (that is, the tendency for individuals in a group to remain closely associated with each other in space) as a measure of their collectiveness. Group polarisation (that is, the tendency for individuals in a group to align their direction of travel with each other) was not measured in these studies. Therefore when referring to groups' collectiveness within this thesis, this only refers to group cohesion. For simplicity, group cohesiveness will be referred to throughout this thesis as 'collectiveness.'

# Data Chapter 1: Investigating interactions between the collective behaviour of groups and habituation to a novel environment in sticklebacks (*Gasterosteus aculeatus*)

## Introduction

There are several antipredator benefits associated with being a member of a group, as has been previously discussed (Elgar & Catterall 1981; Foster & Treherne 1981; Morgan & Godin 1985; Lima & Dill 1990; Ruxton *et al.* 2007; Ioannou *et al.* 2008). In order to remain part of a cohesive and coordinated group, individuals must synchronise their decision making with that of other group members. However, if individuals blindly follow the decisions of other group members, this can lead to an informational cascade, and result in a poor decision being taken by all members of a group (Dall *et al.* 2005). If individuals never bias their decision-making toward that of other group members (i.e. only make decisions based on private information), they fail to exploit the potential fitness benefits available from effectively utilising social information, and the group may undergo fission.

There have been a number of mechanisms proposed to explain how individuals within a group utilise personal and social information, and come to a decision that maximises their fitness. The mechanisms that underlie consensus decision-making can vary between species and contexts. For example, groups may make 'unshared' decisions to come to a consensus, through mechanisms such as despotism (where individuals copy the decisions of leaders) (Conradt & Roper 2003). On the other hand, groups may make 'shared' decisions, whereby all individuals contribute to the decision (for a review see Conradt & Roper 2005; King & Cowlishaw 2009). Several studies have shown that in groups of fish (and many other organisms that form groups), a form of shared decision making

tends to occur, known as quorum-decision making. This is a simple rule, whereby an individual's tendency to make a particular behavioural decision increases with the proportion of other conspecifics who have made the same decision (Ward *et al.* 2008).

When making a movement-based decision, using quorum decision-making allows groups to come to a consensus, and prevents the group from splitting. This is seen in Halloy *et al.* (2007), where cockroaches tended to reach a consensus when choosing which of two refuges to shelter under, thereby preventing the fission of the group between two refuges. Furthermore, Halloy *et al.* (2007) and others (Ward *et al.* 2008) have demonstrated that this decision making process can be manipulated by the 'decisions' of man-made replica conspecifics that are controlled by the researcher, providing an effective method of studying these aspects of collective decision making in the future. This study will investigate whether groups of sticklebacks show evidence of consensus decision making when traversing the novel environment, by examining whether groups tend to all utilise one refuge at the same time, or whether they show no preference for coming to a consensus, and tend to utilise two separate refuges at the same time.

Using a refuge may benefit an individual by reducing their immediate risk of predation (Cowlishaw 1997; Sih 1997); however, there are also costs associated with using refuges. Individuals may lose out on feeding opportunities as a result of spending time within a refuge, rather than foraging (Krause *et al.* 1998). Therefore, if an individual spends a prolonged period of time within a refuge when the actual predation risk is low (and therefore, the defence provided by a refuge is not required), that individual suffers a potential cost to fitness, through the loss of feeding opportunities. This suggests that an individual should only use a refuge when there is a high risk of predation, in order to offset the costs of using a refuge with the antipredator benefits that they provide in high-risk situations. As an individual's decision to use a refuge at any given time tends to reflect its perceived risk of predation within an environment, many studies have used refuge use as a proxy to estimate an individual's perceived risk of predation

(Krause *et al.* 1998; Martin & Lopez 2005), or have assumed that leaving a refuge is an action that an individual perceives as high-risk (McDonald *et al.* 2016). This study also uses the assumption that an individual's tendency to use a refuge reflects its perceived level of predation risk at any given time. For example, as an individual habituates to the test environment over time, its perceived risk of predation will decrease, and as a result, we would also expect its tendency to occupy a refuge to also decrease. This change in refuge use forms the quantitative measure of a group's rate of habituation in this study.

In 1999, Lima and Bednekoff created the Predation Risk Allocation Hypothesis (Lima & Bednekoff 1999, for a review see: Beauchamp & Ruxton 2011). This model recognises that the risk of predation in an environment varies temporally and spatially. This can be due to several factors, such as the activity patterns of predators (Fenn and Macdonald 1995), the distance to nearby refugia, and the size of the group of prey (Creel & Winnie 2005). The Predation Risk Allocation Hypothesis states that in order to maximise their fitness whilst also meeting their energy demands, individuals in high risk situations should exhibit strong antipredator behaviours, and allocate less time to foraging; whereas in situations with a low risk of predation, individuals should allocate more effort to foraging, and less to antipredator behaviours. This model should also apply to situations where the *perceived* risk of predation is high or low, regardless of whether there is a real threat of predation or not (for example, in a novel environment where the presence/absence of predators has not been established).

By adapting the predictions of the Predation Risk Allocation Hypothesis to this experiment, we can reach our hypothesis for this study: at the beginning of the trials (when the perceived predation risk is at its highest and groups are not habituated to the novel environment), the exhibition of antipredator behaviour should be at its peak, as the group holds little to no information regarding the predation risk of the novel environment. In the context of the behaviours measured in this study, this will facilitate as groups moving around the novel environment highly collectively and exhibiting a high level of refuge use. However, as the groups habituate to the environment over the course of the trial,

we hypothesise that individuals will reduce their expression of antipredator behaviours, resulting in a reduction in overall collectiveness and less frequent refuge use. Although the Predator Risk Allocation Hypothesis predicts that individuals should forage more frequently in low risk situations, the foraging rate of individuals will not be measured in this study, as there will be no available food resources in the experimental setup.

As collective behaviour facilitates the transfer of social information between group members, our second hypothesis is that groups that behave more collectively at the beginning of the trials will habituate to their environment faster than less collective groups, due to the advantage that sharing social information provides to groups that behave collectively.

# Methods

## Experimental Subjects

Three-spined sticklebacks (*Gasterosteus aculeatus*) were caught from the River Cary in Somerset between September and November 2016. The subjects were held in 90L tanks (70cm x 40cm x 35cm) in a temperature-controlled room (water temperature was 15-17 degrees centigrade), with controlled daily photoperiods of 12 hours. The population were fed a mix of bloodworm (*Glycera sp.*), and crustaceans (*Mysis sp.* and *Artemia sp.*) once daily in the morning, before the trials took place. This experiment used 160 individuals from a larger wild-caught population.

## Experimental Tank

The experimental setup consisted of a narrow trial area (70cm length x 20cm width) within a larger tank (90L, 70cm x 40cm x 35cm) for the trial to take place (see Figure 1). The trial area was built by fixing a false wall within the tank, parallel to the longest side of the tank, creating a narrow area at the front of the tank. The trials were filmed from the front of the tank, using a Panasonic HC-X920 video camera (1080p, 50fps). Within the trial area, there was an identical refuge on either end (18cm deep, 10cm high, 20cm wide) made from black plastic mesh, and an open area (length 32cm) between the two refuges. These refuges were staple-shaped, with mesh covering both of the sides and the top. The back of the refuge was formed by the sidewall of the tank, and the front of the refuge was left open to facilitate the free movement of fish between the refuges and the open area. This formed two shaded areas in the tank, which were considered to offer areas of refuge for the fish. Studies have shown that fish utilise shaded areas as refuges from predation (Helfman 1981; McCartt *et al.* 1997), and shaded areas have been assumed to act as refuges in several other studies (Reebs 2000; Sumpter *et al.* 2008).

At the beginning of a trial, a partition made from opaque Perspex was fixed in place at the midpoint of the open area (which was marked using permanent marker pen on the outside of the glass) using small magnets. This barrier was

removed following an acclimatisation period of 150 seconds after the fish were introduced to the tank.

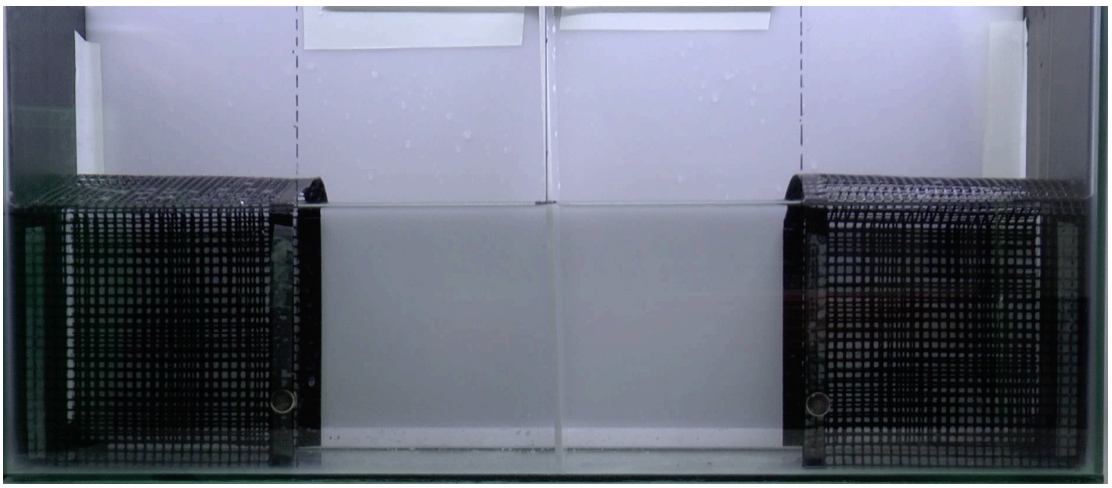

**Figure 1: Experimental Tank Setup. In this photo, the Perspex barrier is in place in the centre of the tank.**

## Experimental Protocol

Groups of eight randomly selected individuals were tested in each trial. The group were introduced to a randomly selected side of the trial area (which was selected using a random number generator), and left to acclimatize for 150 seconds before the central partition was removed, and the trial began. Each trial lasted 30 minutes, during which time the subjects were left to freely move between the two refuges, and around the open trial area. Twenty trials were conducted in total.

## Data Analysis

The statistical tests conducted within this data chapter use asymptotic p-values unless otherwise stated.

The trial videos were analysed using Behavioural Observation Research Interactive Software (BORIS). This is a software programme that allows the user to watch a trial video, and record each occurrence that the focal individuals in the video exhibit specific behaviours of interest. This allows the user to analyse video footage in real time, and produce quantitative data of the behaviours observed during the video. This data can then be downloaded in the form of a spreadsheet, and then analysed using statistical software.

Two sets of 'behaviours' were recorded using the BORIS programme. The first was the number of fish outside of the refuges (i.e. within the open area) at any given time. This variable had a minimum value of 0 and a maximum value of 8. Each trial was observed in real time, and the number of fish that could be observed outside of the refuges was continuously recorded, with any changes in the number of fish outside of the refuges recorded by pressing the corresponding number on the keyboard.

The second variable recorded was the number of fish in each of the two refuges, when all eight individuals were within the refuges (i.e. when none of the fish were in the open area of the tank). In order to create this variable, the trial videos were viewed in real time a second time, and each time all eight individuals were within the refuges, the video was paused, and the number of individuals within the left refuge was counted and recorded by pressing the corresponding number on the keyboard. This variable made it possible to determine whether groups tended to reach a consensus, whereby all individuals would occupy the same refuge, or whether groups tended to split between the two refuges. Collective refuge use would manifest as extreme values of this variable (0 or 8 individuals in the left refuge, depending on whether all individuals were using the right or left refuge, respectively). Weakly collective refuge use would manifest as values in the middle of the range of the variable (around 4 individuals in each refuge), suggesting that individuals do not have a strong preference for using refuges collectively with the rest of the group.

As removing the central partition may have caused a fright response in the fish, influencing the movement and behaviour of individuals at the start of each trial, the first five minutes of each trial was discounted from any analysis. This kind of omission was also conducted in Ioannou *et al.* (2017), in order to remove the influence of fright responses on the analysis of the rest of the trial data.

### *Degree of Habituation*

Using the data acquired from the BORIS software, the mean number of fish outside of the refuges was calculated for the five minutes at the start of each trial

(05:00-10:00, as the first five minutes was omitted from all analyses), and the final five minutes of each trial (25:00-30:00) for each group.

In order to assess the degree to which a group habituated to the novel environment over the trial period, the mean number of fish outside of the refuges between 05:00-10:00 miniutes was subtracted from the mean number of fish outside of the refuges between 25:00-30:00 minutes. This measure allowed us to examine the change in each group's exploration of the open area over the trial period, and thus provides a quantitative measure for the degree of habituation exhibited by each group. If habituation had occurred through the trial, we would expect an increase in boldness between the start and the end of the trial, whereby individuals show a greater tendency to occupy the open area of the tank (which is likely perceived to carry a higher risk of predation than the refuges) as the trials progressed. This would manifest itself as a high 'degree of habituation' value.

### *Initial Collectiveness and End Collectiveness*

Three measures of collectiveness were calculated for each trial. The first, 'Initial Collectiveness', was produced to assess how collectively each group utilised the refuges and the open area between 05:00 and 10:00, relative to all other groups that were observed.

The number of fish outside of the refuges at any given time between 05:00 and 10:00 was transformed several times in order to produce a relative measure of collectiveness. First, the data was folded, so that all values lay between 0 and 4, rather than 0 and 8 (see Table x for details). Before this transformation, a count of 0 would indicate that all eight individuals were within the refuges at that given time, whereas a count of 8 would indicate that all eight individuals were within the open area between the two refuges at that given time. As both of these situations indicate a high degree of group cohesion, this folding transformation removed the difference between these values. This resulted in a scale between 0 and 4, whereby counts indicating a high degree of group cohesion were all given

a value of 0, and higher counts indicated that the group was split between the refuges and the open space.

The mean of these values was taken and then subtracted from 4 to reverse the values so that a higher mean value would indicate stronger, rather than weaker, collectiveness, and these values for each trial were then normalised to range from 0 to 1. This measure of collectiveness was used to determine whether a group's rate of habituation to a novel environment could be predicted by how collectively the group behaves when first introduced to the new environment.

The method that was used to produce the initial collectiveness measure was repeated on the data in the final five minutes of each trial (25:00 – 30:00) in order to produce the term 'end collectiveness'. This term was produced in order to assess whether group collectiveness increased or decreased over the course of each trial.

**Table 1: Initial and transformed values when the 'folding' transformation is applied, to produce the 'Initial Collectiveness', 'End Collectiveness' and 'Within Refuge Collectiveness' terms. The initial value represents the number of fish outside of the refuges at any time during the period of interest (05:00-10:00 for initial collectiveness and 25:00-30:00 for end collectiveness). Values that change after transformation are highlighted. Formula for 'folding' transformation: If $x_1>4$, $x_2=abs(x_1-8)$**

| **Initial Value ($x_1$)** | **'Folded' Transformed Value ($x_2$)** |
|---|---|
| 0 | 0 |
| 1 | 1 |
| 2 | 2 |
| 3 | 3 |
| 4 | 4 |
| 5 | 3 |
| 6 | 2 |
| 7 | 1 |
| 8 | 0 |

### *Within Refuge Collectiveness*

The third measure of collectiveness was calculated from 05:00 - 30:00 of the trial (due to the omission of the first five minutes of the trial from all analyses).

When all eight individuals were within either of the refuges, the number of fish in the left refuge was counted. These counts were then folded, using the same methodology as that used in Table 1. Each occurrence of all eight individuals utilising the refuges at the same time was given a weight, based on the duration that all individuals remained within the refuges. The weighted mean number of fish inside the left refuge when all individuals were utilising the refuges was then calculated for each trial. These weighted means were then flipped and normalised using the same process as in 'Initial Collectiveness'. These transformations produced a term that indicated how collectively each group used the refuges throughout the trial, whereby a lower 'within refuge collectiveness' value indicated that the group were weakly collective when using the refuges (i.e. the group was often split between the two refuges), and a higher value indicates that the group behaved very collectively when using the refuges (i.e. all eight individuals tended to use the same refuge at the same time).

# Results

Figure 2 shows the distribution of the number of individuals in the left refuge during each occurrence of when all fish were using a refuge, for each trial. The distributions were largely either unimodal (with a peak at either x=0 or x=8), or bimodal (with peaks at x=0 and x=8) across groups. This suggests that groups tended to come to a consensus regarding which of the identical refuges to use, with all individuals tending to use the same refuge at the same time.

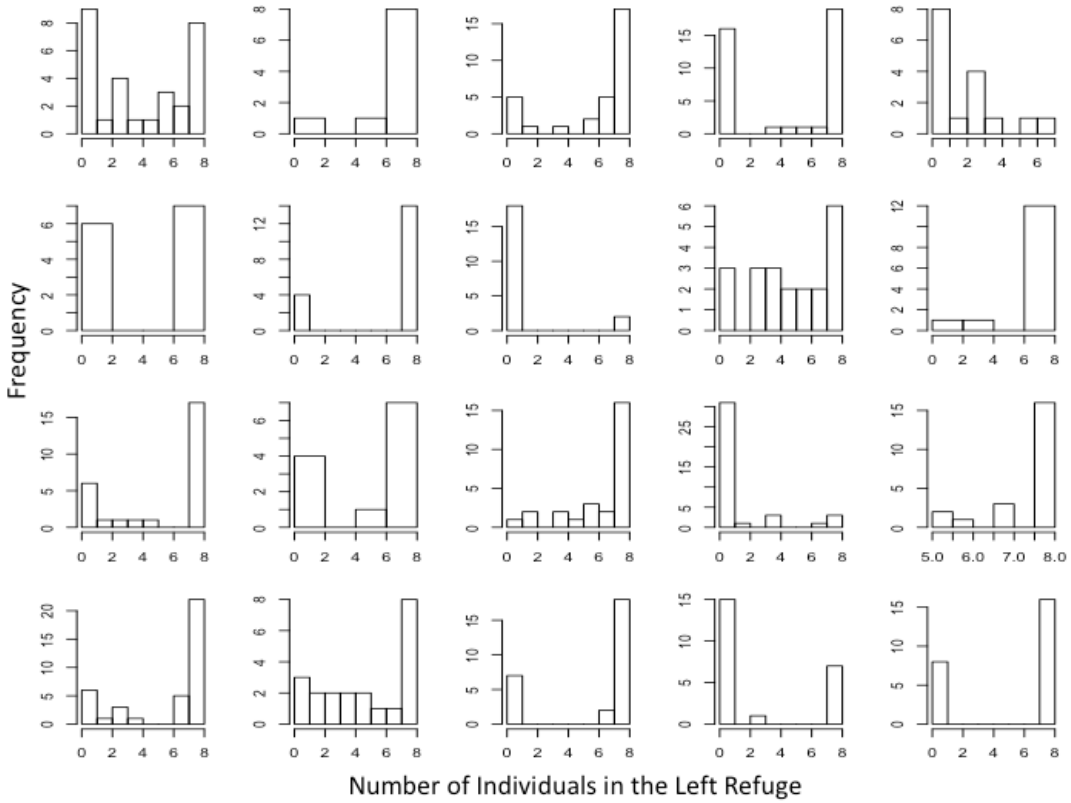


**Figure 2: Histograms showing the distributions of the number of fish in the left refuge when all individuals were using a refuge, for each trial. Note that when x=0, all individuals were using the right refuge, and when x=8, all individuals were using the left refuge.**

A Spearman's rank correlation test was conducted on our 'initial collectiveness' and 'within refuge collectiveness' measures. The two measures were positively correlated with statistical significance (n=20, $r_s = 0.547$, p = 0.013, see Figure 3 below). This indicates that groups that showed a greater tendency to move around the novel environment as a cohesive group during the first five minutes of the trial also tended to use the refuges more collectively throughout the duration of the trial, relative to groups that initially were less cohesive. That is to say, that those individuals within groups that had a higher 'initial collectiveness'

value chose to hide under the same refuge as their conspecifics more often than groups that initially behaved less collectively, and thus remained a more cohesive group throughout the trials.

The fact that there was a significant correlation between these two measures of collectiveness also suggests that there was a degree of variability between groups in how collectively they behaved in the trials. This is important, as without any variability in collectiveness between the trial groups, it would be impossible to make inferences with regard to the interactions between how collectively a group behaves and the degree to which they habituate to the novel environment over the course of a trial.

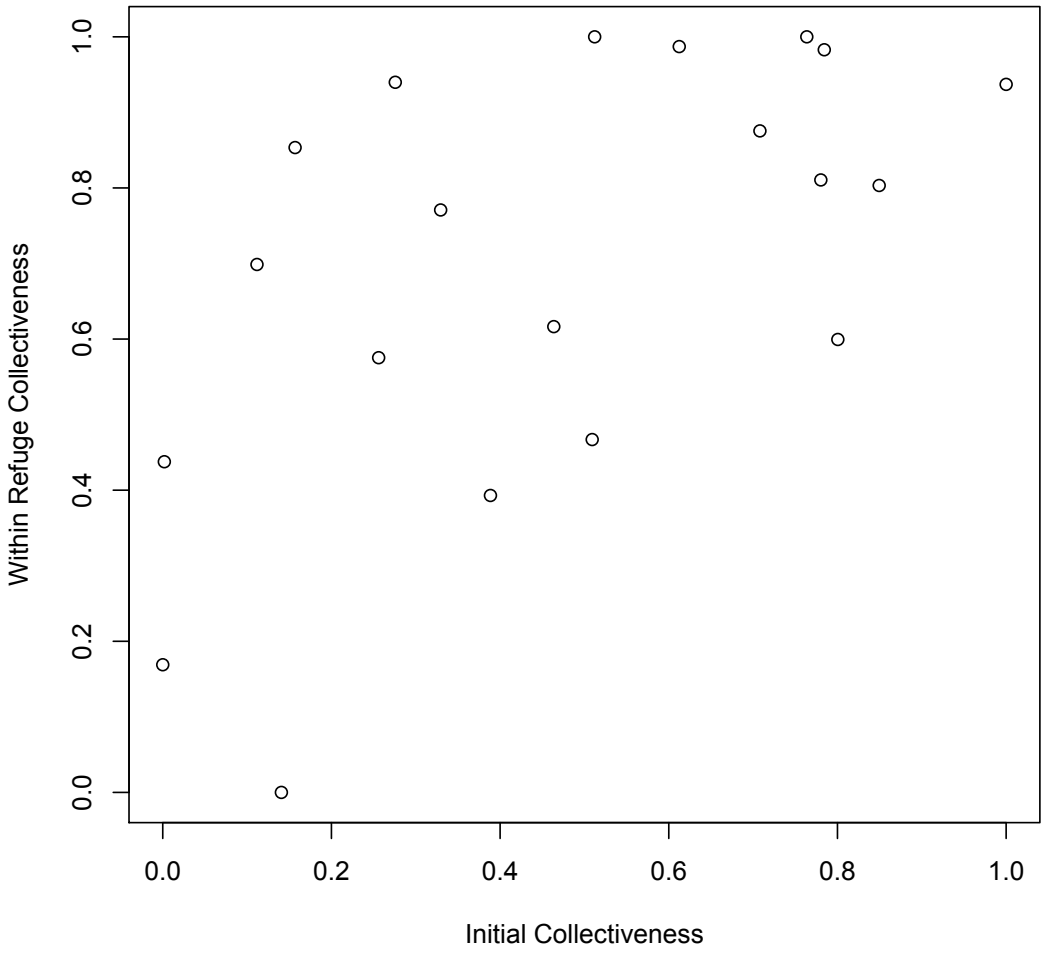


**Figure 3: Scatterplot showing the correlation between 'initial collectiveness' (collectiveness within the initial five minutes of the trial) and the 'within refuge collectiveness' (tendency of individuals within a group to come to a consensus when choosing a refuge) of each group.**

### The interaction between collectiveness and environmental habituation

The initial collectiveness of each group was found to be positively correlated (Spearman's rank: n=20, $r_s$ = 0.474, p = 0.036) with the degree of habituation (that is, the change in the mean number of fish outside of the refuges between the initial five minutes and final five minutes of each trial, or, the change in exploration of the 'risky' open area as the trial progressed) of each group. This suggests that groups that behaved more collectively near the start of each trial habituated to the test environment to a significantly greater degree than less collective groups. This habituation was expressed as a greater increase in the mean number of fish exploring the open area as the trial progressed in groups that behaved more collectively.

However, it appears that many groups did not habituate to the environment over the course of the trials (see Figure 4). Seven groups showed signs of habituation, expressing an increase in time spent exploring the 'risky' open area as the trials progressed, however the remaining thirteen groups exhibited a reduction in the mean number of fish exploring the open area between the beginning and end of the trials (Figure 4). This reduction is the opposite of what would be expected if a group had habituated over the course of the trial, as one would expect a group to spend more time in the 'risky' open area as they habituated to the environment over the course of the trial. Across all groups, there was found to be no significant difference between the mean number of fish outside of the refuges at the beginning (05:00-10:00) and end (25:00-30:00) of each trial (Wilcoxon signed rank test: n=20, V = 60, p = 0.097). However, as this result is only marginally above the significance threshold (as p>0.05), this suggests that a difference between these variables may still be present.

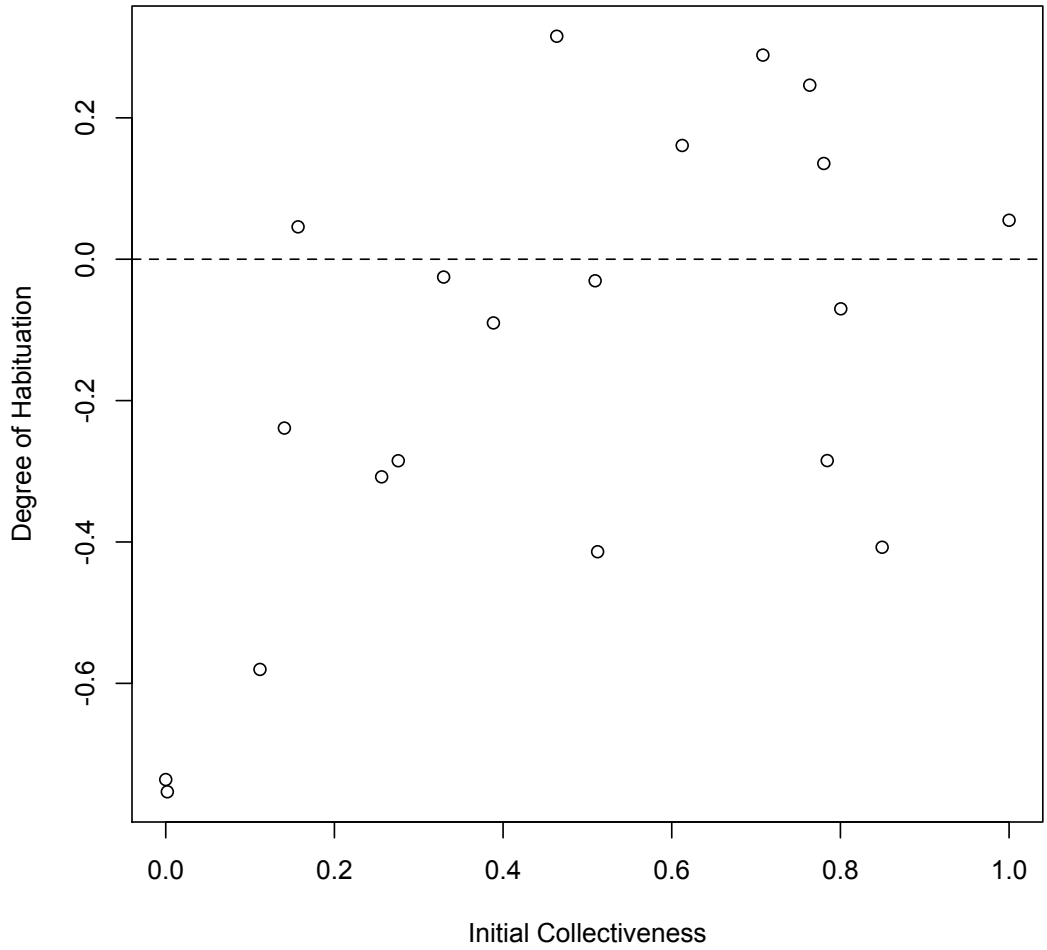


**Figure 4: Scatterplot showing the relationship between a group's collectiveness at the start of the trial and the degree to which groups habituated to the environment. Positive y values indicate that the group showed signs of habituation over the course of the trial (i.e. that there was a positive difference between the mean number of fish outside of the refuges between the beginning and end of the trial).**

Similarly, there was no significant difference between the collectiveness of groups at the start (initial collectiveness) and end (end collectiveness) of the trials (Wilcoxon signed rank test: n=20, V=78, p=0.33), although the median collectiveness was slightly lower at the end of the trials than at the start of the trials (Figure 5). This suggests that groups did not significantly reduce how collectively they behaved as the trials progressed.

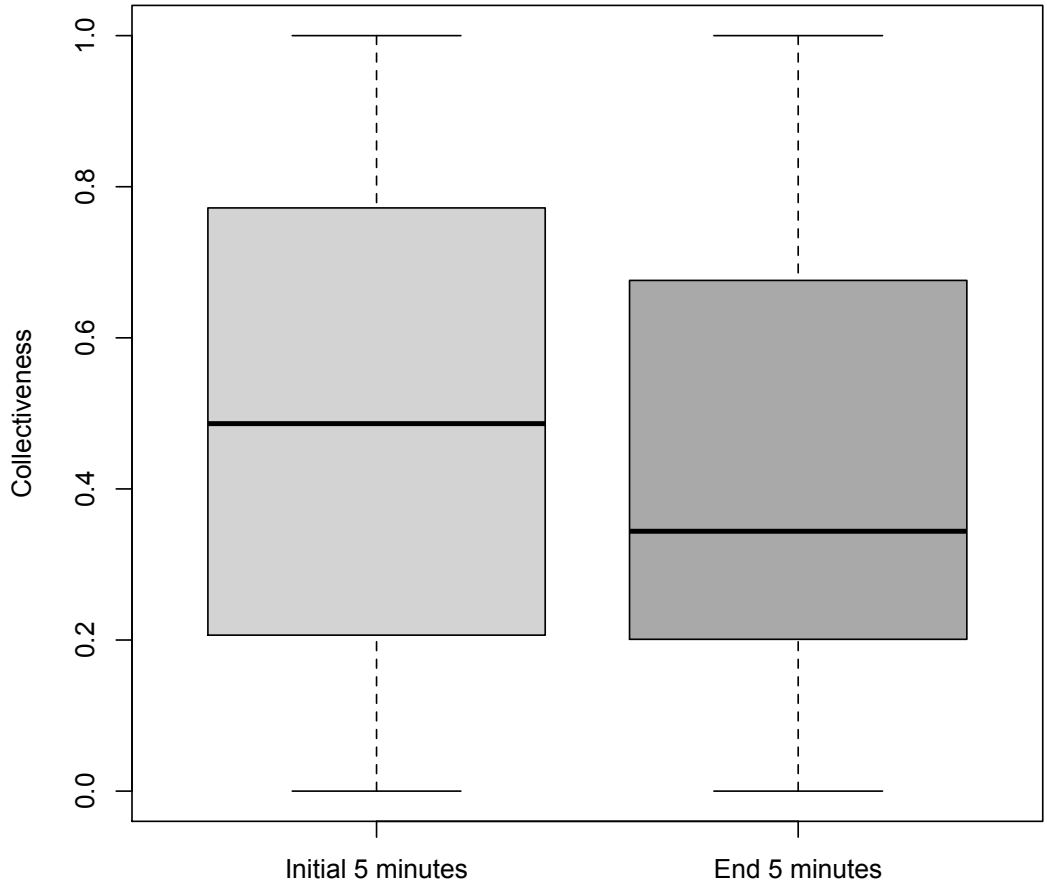


**Figure 5: Boxplot comparing the collectiveness of all groups between the beginning and end of the trials.**

The 'within refuge collectiveness' measure was not significantly correlated with the habituation rate of each group ($r_s$ = 0.354, p = 0.126), or the boldness of each group ($r_s$ = -0.226, p = 0.339). This suggests that the degree to which groups came to a consensus when utilising the refuges was not a good indicator of their rate of habituation, or their tendency to spend time exploring the 'risky' open area of the tank.

# Discussion

## Evidence of consensus decision making

The clear uni-modal and bi-modal distributions of the number of individuals within the left refuge when all individuals were utilising the refuges (see Figure 2) suggests that most groups tended to come to a consensus decision when choosing which of the two refuges to enter. Reaching a consensus decision on which refuge to use prevents a group from splitting between the two refuges. Group fission may reduce the antipredator advantages associated with being a member of a group for all individuals, as effects that reduce individuals' predation risk such as the dilution, detection and confusion effects are all less effective in smaller groups (Roberts 1996; Ioannou *et al.* 2008). Studies have also shown that larger groups tend to make more accurate decisions more often than smaller groups (Sumpter *et al.* 2008), so it follows that a group may make more accurate decisions (the decision that benefits individuals' fitness), and reduce their risk of predation more effectively if its members can reach a consensus and avoid undergoing fission when moving around an environment.

Although this study provides evidence for consensus decision-making in sticklebacks, the mechanism driving this consensus decision-making (that is, whether individuals reached a consensus through following a quorum response rule, whether the decision is unshared and individuals follow particular leaders, or groups follow some other mechanism to reach a consensus) is outside of the scope of this study. However, previous studies have demonstrated that the movement-based decisions of fish are consistent with that of a quorum response (Ward *et al.* 2008). Therefore, it is likely that this is the mechanism that drove individuals' selection of refugia within this study.

## The Interaction Between Collective Behaviour and Environmental Habituation

The within refuge collectiveness measure (how cohesive the group remained when all individuals were utilising the refuges, whereby groups that came to a consensus and all chose to utilise the same refuge were given a high 'within refuge collectiveness' value) was found to be positively correlated with the initial

collectiveness measure (the groups cohesion when utilising both the refuges and the open area), which suggests that both were measuring similar aspects of behaviour (i.e. how collectively a group was behaving). In this context, these are how collectively a group behaved at the beginning of a trial, and how collectively the same group behaved when using the refuges throughout the trial.

The positive correlation between how collectively a group behaved at the beginning of the trial (which was based on the number of individuals outside of the refuges at any given time within the initial five minutes of the trial) and the degree to which they habituated to the novel environment (which was based on the mean difference in individuals outside of the refuge between the initial and final five minutes of the trial) suggests that behaving collectively does offer potential benefits to a group's rate of habituation. An individual's rate of habituation can have fitness consequences (Rodriguez-Prieto *et al.* 2010a), because if an individual's perceived risk of predation is higher than the actual predation risk of a given environment for a prolonged period, individuals may waste time and energy investing in antipredator behaviours (such as using refuges or schooling with conspecifics), when they could be exhibiting other behaviours that benefit fitness, such as foraging. This indicates that behaving collectively (and, consequently, sharing social information with other group members) can offer significant fitness advantages, other than that obtained from directly reducing individuals' predation risk through detection, dilution and confusion effects (Elgar & Catterall 1981; Lima & Dill 1990; Ruxton *et al.* 2007; Ioannou *et al.* 2008), and therefore information transfer may be a driver of the evolution of collective behaviour.

However, although there was considerable variation in how collectively each group behaved, there was found to be no significant difference in the time that groups spent outside of the refuge between the start and end of each trial. Many groups in the study did not exhibit an increase in the time spent in the open area over the course of the trials, and therefore showed no signs of environmental habituation. This could have occurred for a number of reasons. Firstly, the duration of the trials (30 minutes, excluding the acclimatisation period of 2:30 at

the beginning of a trial, before the partition was removed) may have been insufficient for groups to habituate to the environment. Other studies such as Miller and Gerlai (2012) have demonstrated a significant habituation effect in trials of thirty minutes, however, focal individuals were trialed for thirty minutes daily over a series of five days, so the overall duration spent within the novel environment is much higher in Miller and Gerlai (2012) than that used in this study. McDonald *et al.* (2016) also observed a habituation effect when trialling groups over two twenty-minute trials across two consecutive days, which again results in a longer duration that individuals are exposed to the novel environment than the individuals used in this study. Perhaps if the trials in this study were longer, highly collective groups may have showed a greater degree of habituation, demonstrating a greater potential benefit to environmental habituation from behaving collectively. However, it is possible that all groups may have exhibited a higher degree of habituation if the trials were longer, obscuring the potential benefit of behaving collectively on the rate of environmental habituation.

Another potential explanation for the lack of habituation exhibited by some groups is that there was no incentive for groups to extensively explore the open environment. As the open area contained no obtainable resources (such as food patches, refugia, or potential mates), there were no potential rewards for exploring the open environment towards the end of the trials. Once an individual had sampled the environment to the point where it could be concluded that there were no available resources present, the most optimal use of energy was likely for an individual to abort exploring that environment (Lima 1984), and to reduce their overall activity. In environments with food patches, this would be similar in mechanism to an individual abandoning a food patch once it reaches the marginal capture rate, as the energy returns are no longer worth the energy cost of foraging within that patch (Cowie 1977). That is to say, that over the course of the trials, individuals may have become desensitized to the open area, and this could explain the lack of evidence of habituation across many of the groups in this study. It is also rational to conclude that the best areas to be inactive within the test tank were within the refuges, as individuals could still

receive the antipredator benefits that the refuge provides, whilst also conserving energy.

As well as there being no significant difference in the degrees of habituation across all groups in the study, there was also no significant difference in how collectively groups behaved between the beginning and end of the trials. This suggests that, overall, there were no signs of deterioration in collectiveness as the trials progressed. A deterioration in collectiveness would be expected if habituation had occurred over the course of the trial, in line with the findings of Miller and Gerlai (2012), whereby zebrafish tended to more frequently form disorganized shoals over organized schools as they habituated to a novel environment. As exhibiting antipredator behaviour is not necessary following habituation to an environment with no predators present, individuals may benefit the most (in terms of fitness) by behaving less collectively following habituation, spending more time foraging in small groups, or even solitarily, in order to reduce food competition (Hoare *et al.* 2004), however, this phenomenon was not observed in this study.

The most likely explanation for the lack of deterioration of collective behaviour is that groups had not habituated to a significant enough degree within the trial time for the collective behaviour of individuals to be affected. It is possible that, if the trials were longer, groups may have shown signs of deterioration of collectiveness as the trial progressed.

However, another explanation is that, following habituation, groups simply spent less time in a highly coordinated school, and more time in a loosely coordinated, but equally as cohesive, shoal. This was observed in Miller and Gerlai (2012), whereby rather than groups undergoing complete fission following habituation, groups simply became less polarised, and spent more time in a looser configuration. Our data only allowed us to identify whether a group was spatially separated (i.e. whether groups existed across more than one of the environments within the experimental tank) at any time during the trial, and did not allow us to differentiate between levels of polarization (e.g. schooling and shoaling) reliably.

In a further study, tracking software could be used on our trial videos (similar to that used in studies such as McDonald *et al.* (2016), in order to identify the direction that each individual was facing at any time throughout the trial. This would allow us to calculate a group's degree of polarization, and may allow us to reliably differentiate between schooling and shoaling behaviour.

In summary, our data supports the presence of consensus decision-making in sticklebacks, as all individuals within a group tended to preferentially use one of two identical refuges. This study supports the hypothesis the sharing of social information facilitates groups that behave collectively to habituate to their environment to a greater degree than groups that behave less collectively. However, there was no evidence of deterioration in groups' collectiveness as the trials progressed, and our results suggest that habituation did not occur in over half of the groups trialed. Some potential mechanisms that may have prevented groups from showing signs of habituation have been identified and discussed. Further studies could investigate more subtle changes to a group's collectiveness as they habituate to an environment, such as a difference in a group's polarization (that is, their tendency to exist as a school or a shoal) over the course of a trial.

# Data Chapter 2: Investigating the interaction between sociability and environmental habituation in individual sticklebacks (*Gasterosteus aculeatus*)

## Introduction

Data Chapter 1 investigated the interaction between the perceived risk of predation in an environment over time, and how this interacted with the collective behaviour of a group of sticklebacks. The purpose of this first study was to examine whether groups that behaved collectively could utilise social information from conspecifics to habituate to an environment faster than groups that behaved less collectively. However, because local interactions between group members are highly complex and occur at such a fast rate within a school or shoal, it is very difficult to investigate the processes behind information transfer between group members, without the use of individual tracking (e.g. Strandburg-Peshkin *et al.* 2013) or computer-based modelling (e.g. Couzin *et al.* 2005).

It is also likely that there may be factors that produce differences in habituation rates not only between groups, but also between individuals. These factors are also difficult to investigate using a group-based study, such as that used in the first data chapter, as studies have shown that individual differences in behaviour (i.e. personality differences) can be suppressed in a group setting (McDonald *et al.* 2016). This second study will examine these individual behavioural differences, and investigate how behavioural differences between individuals affect each individual's rate of environmental habituation.

Inter-individual differences in behaviour, which are consistent over time and across contexts, are said to constitute aspects of an individual's personality (Bell

2005). Evidence of individual personality has been found in several vertebrate taxa, including birds (Dingemanse *et al.* 2002), mammals including primates, mustelids, dogs, rodents, and livestock, and fish (for a review, see Gosling 2001).

One behavioural trait that has been shown to be consistent within an individual over time and across contexts is boldness. Boldness can be defined as the willingness of an individual to take risks in order to potentially receive greater rewards. The position of an individual on the boldness/shyness axis is linked to its tendency to exhibit a range of behaviours, and these tendencies are consistent across time and contexts (Bell 2005). For example, studies have found that bolder individuals are consistently more predisposed to exploratory behaviour than shyer individuals (Budaev 1997); resume foraging faster than shyer individuals following a predator attack (Webster *et al.* 2007); and are more aggressive towards conspecifics and bolder towards predators (Huntingford 1976). Studies have also demonstrated that bolder individuals habituate faster to a novel environment, such as an experimental tank (Wilson *et al.* 1993). Together, these behavioural tendencies associated with an individual's boldness are known as a behavioural syndrome (Bergmüller 2010).

Ward *et al.* (2004) investigated the presence of boldness as a personality trait in sticklebacks, by examining their behaviour across four social contexts. The study found that individuals that resumed foraging faster after a predator attack also exhibited low motivation to behave collectively. These individuals (who where considered to be bold) exhibited a faster growth rate than individuals that were deemed to be shy, and consistently outcompeted shyer individuals for food. This study demonstrates that sticklebacks show evidence of personality, as individuals varied in their position on the bold/shy axis, and bold and shy individuals exhibited consistent differences in behaviour across several contexts.

This study also demonstrates that inter-individual differences in behaviour can have fitness consequences. By outcompeting shyer individuals for food and exhibiting a faster growth rate, bolder individuals obtained advantages over shyer individuals that directly benefitted their fitness. This poses a question: if

being bold consistently provided fitness benefits across contexts, one would expect natural selection to consistently favour bolder individuals, resulting in the boldness/shyness axis being evolutionarily unstable. However, it seems that boldness may be negatively correlated with an individual's survival rate (Smith & Blumstein 2008), perhaps as a result of the tendency of bolder individuals to take greater risks. There is also evidence that fluctuating environmental conditions, such as availability of food and variation in population density, produces temporal and spatial changes in selection pressure, which may favour either a bold or shy strategy (Dingemanse *et al.* 2004). These studies indicate the presence of a trade-off in fitness consequences between bold and shy strategies that allows a boldness/shyness axis to be evolutionary stable, and hence for inter-individual differences in boldness to be maintained within populations.

Studies have also provided evidence for sociability being a personality trait in certain species. For example, Cote and Clobert (2007) investigated the sociability of common lizards (*Lacerta vivipara*). Individuals in this study exhibited inter-individual variation in their social tolerance (sociability), which was consistent over their lifetime and across social contexts.

Previous studies have demonstrated that personality traits such as boldness and sociability interact in several ways. For example, the boldness of an individual influences the spatial position that it may take up within a group (Ward *et al.* 2004; McDonald *et al.* 2016). The position of an individual within a group conveys certain costs and benefits. For example, individuals towards the middle of a group may receive the best antipredator benefits, as they are less vulnerable to an attack than individuals at the front of a group, but they suffer in terms of potential to forage, as individuals at the front of a group will have first access to discovered food patches. Bolder individuals tend to take up positions at the front of a group (Ward *et al.* 2004), where they receive potentially greater rewards (McDonald *et al.* 2016), but at the cost of a higher risk of predation (Bumann *et al.* 1997). Another interaction between individuals' boldness and sociability traits was demonstrated in Kurvers *et al.* (2010), whereby bolder barnacle geese (*Branta leucopsis*) were found to utilise available social information to a lesser

extent than shyer individuals. Previous studies have suggested that shyer individuals pay closer attention to the behaviour of nearby conspecifics, and therefore may collect more social information and utilise it to a greater extent than bolder individuals (Stowe & Kotrschal 2007; Harcourt *et al.* 2009; Kurvers *et al.* 2010), however this is disputed by other studies, which suggest that variation in boldness does not affect individual's use of social information (Harcourt *et al.* 2010). As studies have found that sociability and social tolerance is a personality trait (Cote and Clobert 2007; Rodriguez-Prieto *et al.* 2010b), and there is some evidence that an individual's boldness is related to their propensity to utilise social information (Kurvers *et al.* 2010), it is also likely that an individual's sociability may be related to their propensity to utilise social information.

Studies have also shown that individuals within a population vary in their innate ability to habituate to stimuli (Runyan & Blumstein 2004; Ellenberg *et al.* 2009). It is likely that this habituation ability forms an aspect of a behavioural syndrome that is tied to an individual's personality traits, such as boldness and sociability (Rodriguez-Prieto *et al.* 2010b). This second data chapter used an individual-based design, with one focal fish per trial, in order to investigate the interactions between an individual's sociability and its rate of habituation to a novel environment. This individual-based design also allowed us to investigate the effect of the boldness personality trait on these factors.

The trials within this study occurred over three consecutive days. On the second day, a small group of conspecifics was introduced to an enclosure within the open area of each trial lane, so that the focal individual could see and approach the conspecifics within the open area, but not directly interact with them. Although the focal individual could not interact with the conspecifics, an individual's sociability could be assessed through the time that it chose to be in close proximity to the conspecific group.

It is likely that the presence of the conspecifics within the open area would also produce social information that the focal individual could receive and interpret.

For example, the presence of conspecifics within the 'risky' open area permanently throughout the trial on Day 2 may have acted as a social cue, and be interpreted by the focal individual as an indicator of the safety of the open area. This is an interesting aspect of 'information transfer', as the term is normally applied to instances where informed individuals generate signals or cues (intentionally or otherwise), which are then transferred to uninformed individuals. In this case, the information that the 'informed' conspecifics within the enclosure possessed (i.e. the safety of the open environment) was artificial, as the group was kept within an enclosure in the open area. This allowed us, to a degree, to generate social cues that the focal fish could receive and interpret. Highly sociable individuals (i.e. individuals who spent the most time around the conspecific shoal on Day 2) were more likely to be picking up on and utilising the cues presented by the presence of the conspecific shoal within the open area, as they were within a closer proximity to the shoal for a greater duration.

Our main hypothesis for this study was that individuals who exhibited the highest tendency to socialise with the conspecific shoal on Day 2 of the experiment would show the greatest degree of environmental habituation over the course of the three trials. This would suggest that the information transferred to the focal individual whilst in proximity to the conspecific shoal (i.e. the positional cue of the shoal within the open area) facilitated a faster rate of environmental habituation than that expressed by other focal individuals who exhibited a lesser tendency to socialise, and therefore utilised the available social information to a lesser extent.

As this study was completed over three consecutive days, this design allowed us to also investigate whether individuals' showed consistent levels of boldness/shyness over the duration of the trials. As well as investigating the relationship between an individual's sociability and its rate of habituation, this study also investigates whether an individual's tendency to explore the open area was consistent over the course of the trials. If this were the case, this would provide further evidence for boldness being a personality trait in sticklebacks. Further to this, we also investigate whether bolder individuals tended to

habituate to the novel environment faster than shyer individuals. From the findings of previous studies, we hypothesise that individuals that are measured to be bolder will be significantly less social than shyer individuals (i.e. spend the least time around the conspecifics on Day 2) in line with the findings of Ward *et al.* 2004), and will also habituate to the environment to a significantly greater degree than shyer individuals over the course of the three trials (in line with the findings of Wilson *et al.* 1993).

## Methods

### Experimental Subjects

Three-spined sticklebacks (*Gasterosteus aculeatus*) were caught from the River Cary in Somerset between September - November 2016. The subjects were held in 90L tanks (70cm x 40cm x 35cm) in a temperature-controlled room (water temperature was 15-17 degrees centigrade), with controlled daily photoperiods of 12 hours. The population used in this study were fed bloodworm (*Glycera sp.*) once daily, following the completion of all of the day's trials. This experiment used 40 individuals from a larger captive population.

### Experimental Tank

Five tanks were used to house the experimental subjects each week. A breeding net (a small enclosed net that allows you to isolate specific individuals within a larger tank) was set up at the front of each tank, which was used to house the focal fish for each week. The tanks also contained other conspecifics (between 4 and 6), which were outside of the breeding nets. These conspecifics were not trialed during this study.

The experimental tank was 137cm long and 72cm wide, and was split in to two identical lanes measuring 36cm in width, which were separated by an angled partition. Two identical lanes were used for efficiency when running trials (as this allowed us to run two trials simultaneously), and this design was not relevant to the aims of the study. The partition between the two lanes was opaque in order to ensure that individuals could not see or obtain information from individuals in the adjacent lane. This partition was made from two pieces of

Perspex plastic, which were fixed together at an angle, so that the bases were 2cm away from the midline of the partition. This was done to minimize the blind-spots of the single overhead camera when filming trials. The tank was in a temperature-controlled room, and the water was maintained at a temperature of 15-16 degrees Celsius.

One refuge was set up in a central position on the far wall of each lane, 11cm from each sidewall. The refuge was made from black corrugated plastic and was fixed in position by small magnets on the wall and the refuge. The refuges were 10cm wide, 8cm high, and 10cm deep. As the refuges were removed on day 2 of the trials, magnets were used to ensure that the refuges could be fixed in an identical position when they were replaced.

On the other end of each lane, a transparent cylindrical enclosure (11.5cm high, with a diameter of 10cm) made from a cross section of a 2L plastic bottle was fixed in place with tape. The enclosure was fixed 12cm from the back wall in each lane, in a central position. These enclosures were higher than the water level in order to prevent fish from escaping, and were pierced with small holes to facilitate water flow (and hence odour cues) with water in the enclosure. Figure 6 shows the design of the experimental tank with the refuges in place.

The tank was on a surface with a slight slope, but there was a constant water level of 10cm at the refuge end of the tank and 7cm at the cylinder end throughout the trials. The entire tank was illuminated by two tube lights (Masterlite fluorescent 13W linkable cabinet light, 585mm in length), which were fixed in place 12cm above each end of the tank. Trials were recorded using a GoPro Hero Session camera that was fixed in place 80cm above the tank on a camera mount.

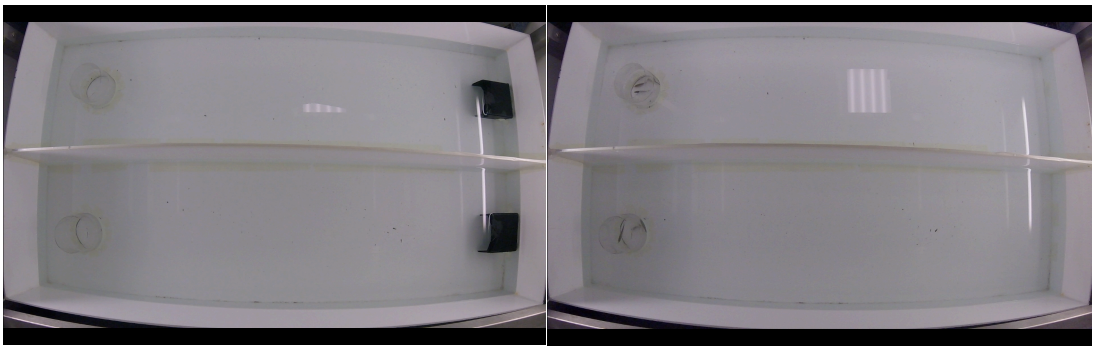

**Figure 6: Still images taken from trial videos. The first image (left) depicts the experimental set-up on Day 1 and Day 3 of the trials with the refuges in place, and the second (right) depicts the set-up on Day 2, with the refuges removed and conspecifics placed in the enclosures.**

## Experimental Protocol

On a Monday, individuals were randomly selected from a larger population of sticklebacks, and pairs of fish were placed in to each of the five breeding nets. Fish with noticeable differences in size were paired together within a breeding net, and each individual in a pair was assigned either as 'L' or 'S' (Large or Small), depending on its size relative to the other individual in its pair, as this made it possible to identify each individual within the same breeding net. This was important, as individuals had to be recognizable in order to be trialed over three consecutive days. Each pair was assigned a breeding net that was kept constant over the course of the three days that they were trialed. Fish within the five holding tanks were fed as normal on a Monday, but food was only provided after trials had taken place on the trial days (Tuesday, Wednesday and Thursday).

### Day 1 and Day 3

On the first day of trials (Tuesday), a pair of fish was taken from a breeding net, and each individual was placed in to one of the two lanes in the experimental tank. The lane that each fish from a pair was placed in to was randomly allocated by the flip of a coin (with a 'heads' resulting in the larger of the two fish being placed in the first lane). The fish were placed in to the open area of their lane using a small net. There were no fish present within the cylindrical enclosure on Day 1 and Day 3 of trials (See Figure 7). Screens made from corrugated plastic were then placed over the trial area in order to minimise disturbance to the fish for the duration of the trial.

Each trial was recorded for 21 minutes; however, the first minute of each trial

video was discounted from analysis, as the fish were introduced to the tank within this period. This also allowed some time for the fright responses that can be induced when handling the individuals to subside. Following completion of the trial, the pair of focal fish was translocated back to their assigned breeding net within the holding tanks.

### Day 2

On Day 2 of each week of trials, the refuges were removed from each lane. Three randomly selected fish from a larger population were placed in to each of the cylindrical enclosures on Day 2 of each week. These were placed in to the enclosures at the start of each day, and were left to acclimatise to the test tank for around 30 minutes before any of the day's trials began. One test fish was then introduced to each lane, following the same procedure as that used on Day 1 and Day 3. Each trial on Day 2 also lasted 21 minutes, to allow the focal fish to be introduced to the tank and for the initial fright response to subside. Ten individuals (from five pairs) were trialed each week, with a total of 40 individuals being trialed over the course of the study.

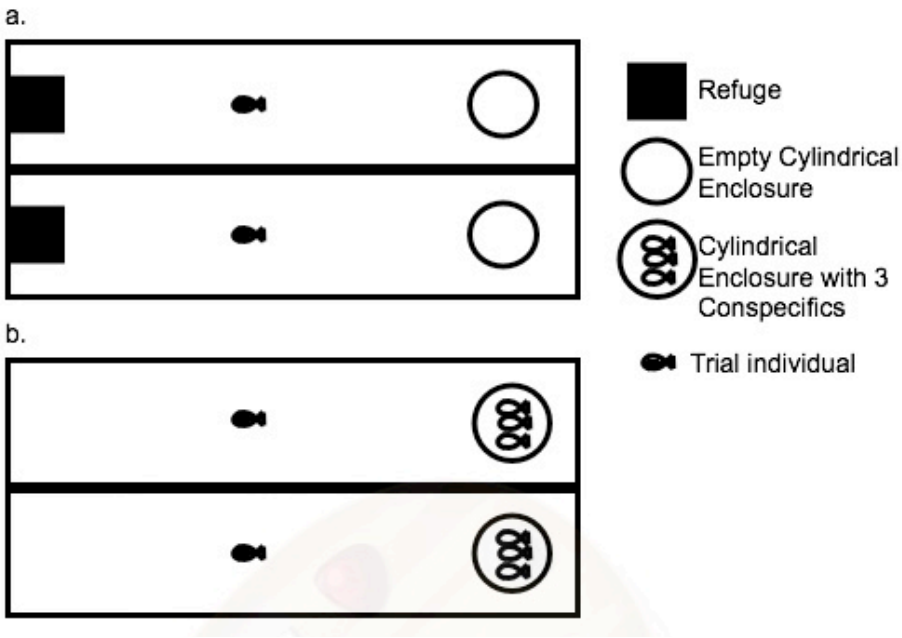


**Figure 7: Illustration detailing the setup of the experimental tank throughout the trials. Format (a.) represents the setup that was used on both Day 1 and Day 3 of trials, and (b.) represents the setup used on Day 2.**

## Data Analysis

The statistical tests conducted within this data chapter use asymptotic p-values unless otherwise stated.

### Day 1 and Day 3

The trial videos were analysed following the completion of all trials. The videos were viewed at 2x speed on iMovie software on a laptop. For videos from Day 1 and Day 3 of the trials each week, the duration that each individual spent outside of the refuge within their lane was recorded. This was recorded by eye, using two stopwatches simultaneously; one for each fish in either lane of the test tank.

To produce a quantitative measure of an individual's degree of habituation over the course of the three trials, the time that an individual spent outside of the refuge on Day 3 was subtracted from their time spent outside of the refuge on Day 1.

### Day 2

During data analysis, the duration that each fish was within one body length of the cylindrical enclosure (containing the three conspecifics) on Day 2 was recorded. The proximity to the cylindrical enclosure was estimated by eye. This was used as a measure of how sociable each individual was on Day 2 of the trials, whereby fish that spent more time close to their conspecifics within the enclosure were deemed more sociable than those that spent more time in other areas of the tank, away from their conspecifics.

### Measure of Individual Boldness

The average time spent outside of the refuges was calculated for each individual, from the time that they spent outside of the refuges on Day 1 and Day 3 of trials. This was used as a basic quantitative measure of an individuals' relative boldness over the course of the trials, whereby individuals that on average spent more time exploring the 'risky' open area on Day 1 and Day 3 were deemed to be bolder than those that spent more time within the refuges on these days.

# Results

## Testing the key assumption of higher perceived risk in open areas

The assumption that sticklebacks find open areas inherently more 'risky' than refugia was a key assumption of this study, as well as in the first data chapter. This assumption was tested by investigating the mean proportion of the total trial that individuals spent in both environments within the test tank. The mean percentage of the trials that individuals spent within the refuge was 53.4% and 62% for Day 1 and Day 3 respectively. Given that the area within the refuge only constituted a small proportion of the total area of the test lane that each individual had access to (the rest of which comprised the open area), this suggests that individuals did spend a disproportionate amount of time within the refuge in comparison to that which would be expected if both environments were seen as homologous, indicating that the focal fish likely did perceive the open areas as inherently more 'risky' than the refuge.

## Was an individual's sociability a good predictor of their degree of habituation?

There was found to be no significant correlation between individuals' degree of habituation to the open area (the difference in time spent outside of the refuges between Day 1 and Day 3), and the time each individual spent in close proximity to the conspecific group within the enclosures on Day 2 (Spearman's rank, n=20, R=-0.006, p=0.973). This suggests that the sociability of individuals was not a suitable indicator of the degree of habituation that they exhibited over the course of the trials.

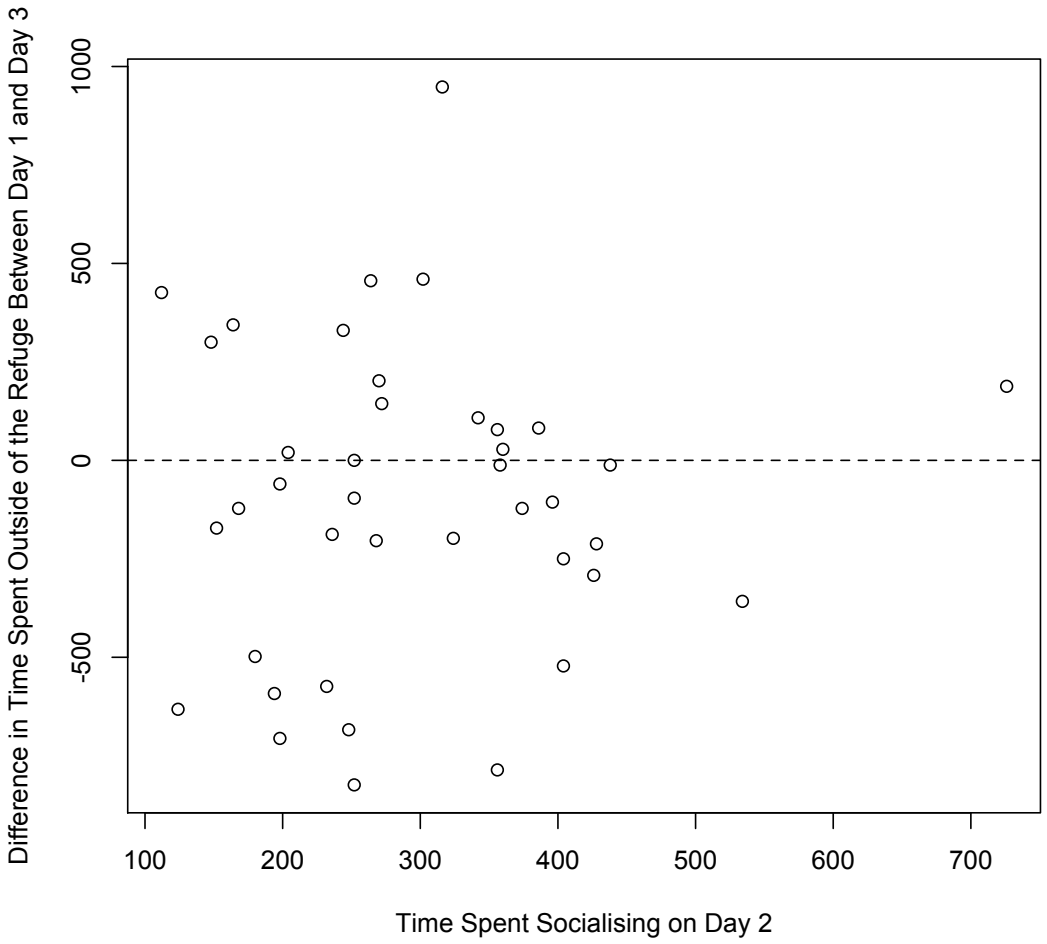


**Figure 8: The change in time spent outside of the refuge between Day 1 and Day 3, relative to the sociability of each individual on Day 2. Note that positive y values denote an increase in time spent outside of the refuges between Day 1 and Day 3 (indicating a degree of habituation), and negative values denote a reduction in time spent outside of the refuges between Day 1 and Day 3.**

Figure 8 shows that individuals that exhibited similar levels of sociability (that is, individuals who spent a similar duration of the trial on Day 2 in close proximity to the enclosure with the conspecifics inside) displayed a large degree of variation in the degree of habituation that they exhibited between Day 1 and Day 3. Figure 9 displays the variability in the response variables between individuals across Day 1, 2, and 3 of the trials. It is clear that there was a large degree of variation in the proportion of total trial time that individuals spent outside of the refuge on both Day 1 and Day 3 (std (Day 1) = 0.337; std (Day 3) = 0.318). However, it is also clear that the degree of variation in time that individuals spent with their conspecifics on Day 2 was much smaller (std (Day 2) = 0.101). A Brown-Forsythe test was conducted to establish whether the variability between the sociability of individuals and the difference in time that they spent outside of

the refuges on Day 1 and Day 3, were equal (Brown & Forsythe 1974). The variance in the sociability of individuals was found to be significantly lower than the variance that existed between the boldness of each individual during Day 1 and Day 3 (F=6.478, DF = 2, p=0.002). This suggests that the individuals sampled within this study were relatively analogous in terms of their sociability, but varied much more significantly in their individual boldness. It seems that this combination of high variability in individual boldness and low variability in individual sociability may have contributed to the lack of a significant correlation between individual's sociability and the degree to which they habituated to the environment over the course of the trials.

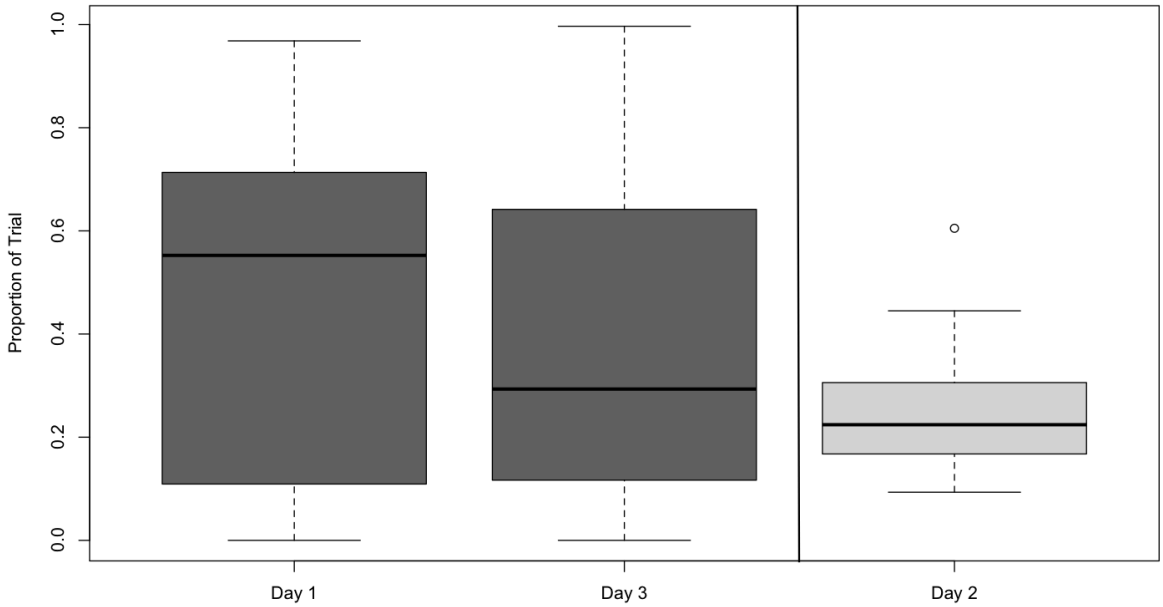


**Figure 9: Boxplot showing the variation in the proportion of the total trial time that individuals spent outside of the refuges on Day 1 and Day 3, and socializing with their conspecifics on Day 2.**

The high degree of variability in the time spent outside of the refuges on Day 1 and on Day 3 also suggests that some individuals may not have habituated to the novel environment to a significant degree over the course of the trials (as some individuals spent very little time within the 'risky' open area on both Day 1 and Day 3). A paired t-test was carried out on the time that each individual spent outside of the refuge between Day 1 and Day 3 of trials. The purpose of this analysis was to discern whether individuals significantly increased the time that they spent outside of the refuges over the testing period, which would suggest that habituation to the test environment had taken place. Across all individuals, there was no significant difference between the time spent outside of the refuges

on Day 1 and Day 3 (Paired t-test, t=1.670, DF=39, p=0.103).

This suggests that some of the sampled individuals had not habituated to the test environment. This is supported by Figure 8, which shows that fifteen individuals spent more time outside of the refuge on Day 3 than Day 1 (suggesting that they may have habituated to the environment to some degree), but twenty-five individuals spent either the same amount of time (n=1), or less time (n=24) outside of the refuges on Day 3 than on Day 1, demonstrating that over half of the individuals trialed did not exhibit signs of habituation between Day 1 and Day 3.

Figure 9 also demonstrates that the median proportion of the trial spent outside of the refuge across all individuals is lower on Day 3 than on Day 1. The opposite trend would be expected if habituation had occurred, as one would expect individuals to, on average, spend a higher proportion of the trial outside of the refuge on Day 3 than Day 1.

### Evidence of boldness being a personality trait in sticklebacks

The time that each fish spent out of the refuge on Day 1 and Day 3 was strongly positively correlated (Spearman's rank, n=20, R=0.542, p<0.001). This significant correlation indicates that individuals tended to be relatively consistent in their choice of whether to spend their time within or outside of the refuges across Day 1 and Day 3. Figure 10 below shows two interesting points that both support the presence of a boldness-shyness continuum within the sampled population. Firstly, there was considerable variation between individuals in the proportion of the trials that they spent outside of the refuges on Days 1 and 3, suggesting that inherently bold and shy individuals existed within the sample population (this variability is also seen in Figure 9); and secondly, that individuals were relatively consistent in the proportion of the trials that they spent outside of the refuge across Day 1 and Day 3, indicating that individuals were *consistently* bold or shy over the trial period.

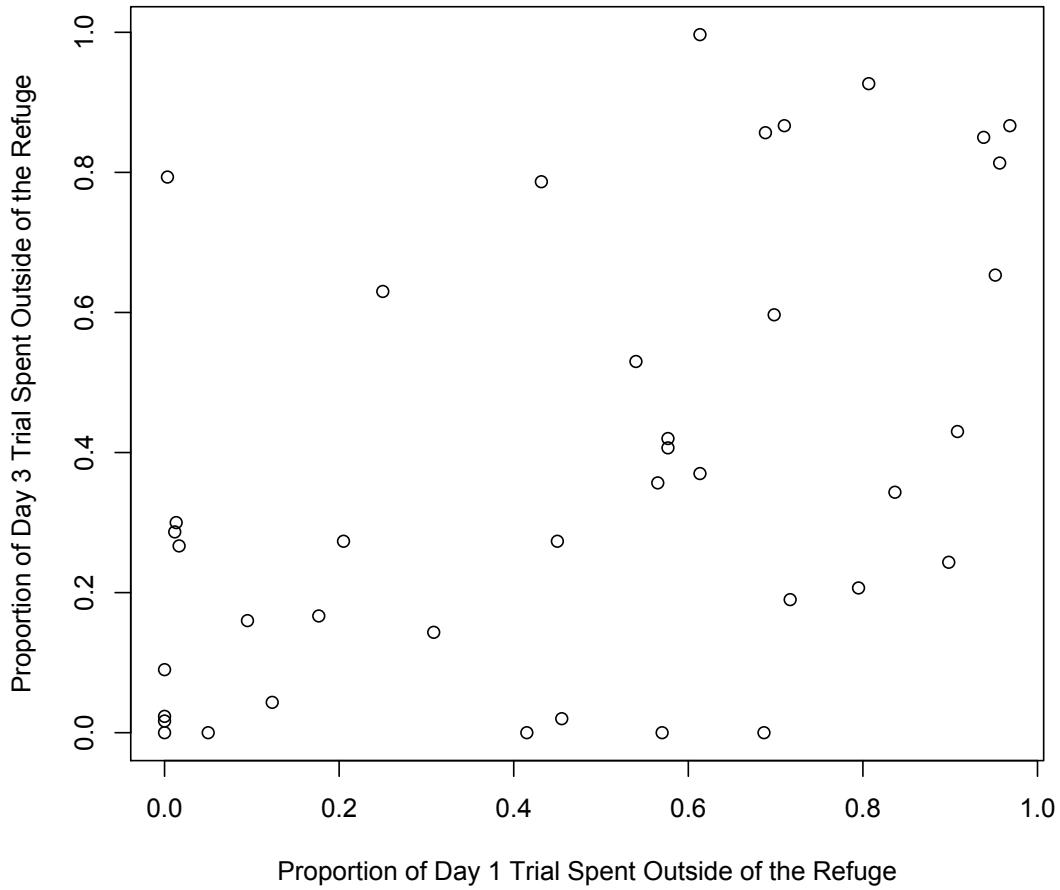


**Figure 10: Scatterplot showing the correlation between the proportion of time individuals spent outside of the refuges on Day 1 and Day 3.**

As the boldness of individuals was found to be consistent over time, we also tested for correlations between the boldness of individuals and their sociability (i.e. the time spent in close proximity to the conspecific shoal on Day 2); as well as between an individual's boldness and the degree of habituation that they exhibited. However, an individual's average boldness across Day 1 and Day 3 was not significantly correlated with their sociability on Day 2 ($R=0.105$, $p=0.518$), or with the degree of habituation that they exhibited ($R=-0.151$, $p=0.354$).

# Discussion

## Testing the Key Assumption of Open Areas Being Associated With a Higher Perceived Risk of Predation

Focal individuals spent on average over half of their time within the refuge, rather than the open areas of the tank, on both Day 1 and Day 3 of the trials. The assumption that open areas are perceived as carrying a higher risk of predation than refugia underpins many previous studies on sticklebacks in behavioural ecology. For example, McDonald *et al.* (2016) used the assumption that the act of sticklebacks crossing an open arena to reach a food source would be a more risk-prone behaviour than initially leaving a refuge, as individuals are more exposed and vulnerable to predation within an open area than when close to a refuge. Similarly, Harcourt *et al.* (2009) and Ioannou *et al.* (2008) both quantified the boldness of individual sticklebacks by their tendency to leave a refuge and enter an open area. Given that the use of refugia and open areas is such an established concept in behavioural ecology, and that the individuals trialed in this study occupied the refuges (a very small proportion of the potential area that could be occupied) for on average over half of the total trial time on both Day 1 and Day 3, it is considered likely that the individuals in this study did find the open space more inherently risky than the refuges, and therefore the use of this assumption in this study was reasonable.

## The Interaction Between Individual Sociability and Habituation

Our key hypothesis stated that highly sociable individuals would reduce their perceived risk of the open environment at a relatively faster rate than less sociable individuals. The main mechanism that was considered was that highly sociable individuals would receive and utilise the social cues obtained from the location of the conspecific shoal within the open environment on Day 2 to a greater degree than less sociable individuals, and thus would reduce their perceived risk of the open environment to a greater degree than less sociable individuals, who would not have had access to this social information to the same extent. However, this study found no significant correlation between individuals' sociability and the degree to which they habituated over the course of the trials. The lack of variability in individual sociability on Day 2 may be a contributing factor to the lack of a significant correlation between individuals'

sociability and their rate of habituation, as correlation tests require a reasonable degree of variability in both variables to produce significant results.

Our results also suggest that many of the individuals trialed did not habituate to the novel environment over the course of the trials. This was indicated by the lack of a significant difference between the time that an individual spent outside of the refuge between Day 1 and Day 3, as well as the fact that twenty-five of the forty individuals in this study either spent the same amount of time (one individual), or less time (twenty-four individuals), outside of the refuges on Day 3 than on Day 1. This makes it difficult to draw conclusions about the relationship between individual's sociability and their rate of environmental habituation, as the number of individuals who did show signs of habituation in this study was too few to produce statistically significant correlations and analyses between our variables. However, it is worth noting that the difference between the time that individuals spent outside of the refuge on Day 1 and Day 3 had a relatively low p-value (paired t-test, $p=0.103$), which may indicate that a trend is present, but was not statistically significant in this study. Given that our data is relatively noisy, it is possible that a study with a larger sample size, for example, would reveal a significant difference between these two variables.

One potential reason for the lack of habituation to the novel trial environment is that the duration of each trial was too short, at twenty minutes per trial. Although each individual was exposed to three twenty minute trials (resulting in each individual having sixty minutes total within the novel environment) over three consecutive days, it is possible that, were each of the trials longer, a higher degree of habituation would have been observed over the duration of the study. For example, Miller and Gerlai (2012) observed a habituation effect in zebrafish (*Danio rerio*) over the period of a continuous trial that lasted four hours, as well as over a series of five daily trials, which lasted for thirty minutes each. In both of these methodologies, the focal fish were exposed to the novel environment for a longer duration than the focal fish used in this study, and thus had more time to habituate to the environment. Although Miller and Gerlai (2012) used a different species of fish with a different experimental procedure, it is still likely that

individuals in this study may have exhibited a greater degree of habituation over a longer trial period, and further studies should take this in to account. The groups of individuals trialed in the first data chapter did show some signs of habituation over a single trial period of thirty minutes; however, these individuals were tested in groups (rather than solitarily, as in this data chapter), and as such, it is difficult to deduce, with any certainty, whether a trial length of thirty minutes would have produced a higher degree of habituation in this study.

Another potential explanation for the lack of an increase in exploratory behaviour over the course of the trials (and, indeed, the observed *reduction* in exploratory behaviour across the course of the trials in twenty-four of the forty individuals trialed) is that the individuals had explored the novel tank sufficiently on Day 1 and Day 2 to deduce that the open area contained no available resources. As no food stimuli were presented within the tank at any time during the trials, a desensitization effect similar to that potentially present in our first data chapter may have occurred, whereby individuals explored the open area until they had concluded that the environment was bare of resources, at which point they reduced their activity within the open area in order to avoid inefficient use of energy (Lima 1984).

In future studies, a larger, stochastic environment with exploitable resources, such as food patches, could be used in order to provide a reward for exploratory behaviour of the 'risky' open area over the course of the trial. A similar methodology was used in McDonald *et al.* 2016, whereby bloodworms were released in to the open area of the experimental tank once the focal individuals had left a refuge and crossed the open area, thus providing a food reward to incentivize exploratory behaviour. The inclusion of a food incentive for foraging individuals would reduce the possibility of a desensitization effect occurring, such as that explored above, and it is possible that individuals would increase their exploration of the open area as they became habituated to the test environment, as was expected in this study. Nevertheless, this study provides little evidence to support the hypothesis that sociable individuals can utilise social information to habituate to an environment faster than less sociable

individuals.

### Evidence for boldness as a personality trait in sticklebacks

The data collected within this study does provide some support for boldness being a personality trait in three-spined sticklebacks. The strong positive correlation between the time that each individual spent outside of the refuge on Day 1 and Day 3 demonstrates that individuals exhibited personalities, defined as inter-individual variations in behaviour that are consistent over time and across contexts, over the course of the three days. Some individuals trialed were consistently bolder than others, exhibiting a greater tendency to spend time in the risky open environment across both Day 1 and Day 3. This result suggests that an individual's position on the bold-shy continuum was relatively consistent over time, under similar experimental conditions. This is in line with the findings of other studies (Ward *et al.* 2004; Ioannou *et al.* 2008), and indicates that boldness is a personality trait in sticklebacks.

Other studies have found that the boldness of an individual is linked to its tendency to exhibit a range of other behaviours, and these tendencies make up a behavioural syndrome that is linked to boldness. For example, in Croft *et al.* 2009, individuals' boldness (defined as an individuals propensity to inspect a predator) and sociability (the time an individual spent shoaling) were negatively correlated, indicating that bolder individuals had consistently lower sociability than shyer individuals (Ward *et al.* 2004). Studies have also shown that bolder individuals utilise social information to a lesser degree than shyer individuals (Kurvers *et al.* 2010), and habituate faster to novel environments (Wilson *et al.* 1993). However, in this study, an individual's boldness was not correlated with their sociability on Day 2 of the experiment, or with their degree of habituation. This study therefore provides no evidence of these aspects of the behavioural syndrome that is associated with an individual's boldness.

As sociability was only tested on one day, this data is insufficient to deduce whether the sociability of an individual was consistent over a period of time or across contexts, and thus we can't deduce whether sociability was a personality trait from our data. However, there has been previous work that suggests that an

individual's sociability is consistent over time, and is therefore a personality trait in some taxa, as has been previously discussed (Dall *et al.* 2004; Cote and Clobert 2007; Cote *et al.* 2010; Rodriguez-Prieto *et al.* 2010b). Future studies could test individual's sociability over a number of days, in order to establish whether sociability is a personality trait in three-spined sticklebacks.

## Conclusion

The data chapters in this thesis used two different approaches to investigate the same question: does behaving collectively affect individuals' rates of environmental habituation? The first data chapter used a group-based design, where individuals within a shoal were able to freely interact with one another. This allowed us to study aspects of collective behaviour, such as how cohesive groups are throughout the process of habituating to the novel environment that they are placed in. We hypothesized that groups that behaved more collectively and that remained more cohesive throughout the trial would exhibit a greater degree of habituation to the novel environment, and that this would manifest as a greater increase in the duration that groups spent outside of the refuge, relative to less collective and cohesive groups.

This study found a positive correlation between groups' collectiveness and the degree to which they habituated to the open environment. The rate at which individuals or groups habituate to environments can impact their fitness (Rodriguez-Prieto *et al.* 2010a), as investing time and energy exhibiting antipredator behaviours (such as sheltering within refuges and forming polarized schools) in environments with no real threat of predation results in the loss of potential foraging time. There are several means by which behaving collectively can benefit an individual's fitness, with most of these effects relating to reducing individuals' predation risk (for example, through detection, dilution and confusion effects (Elgar & Catterall 1981; Lima & Dill 1990; Ruxton *et al.* 2007; Ioannou *et al.* 2008)). However, this study provides evidence for another means by which behaving collectively can benefit fitness. By reducing individuals' rates of environmental habituation, behaving collectively can increase individuals' energy and time efficiency, and result in a potential benefit to their fitness. The main mechanism that was considered to drive this trend was that of information transfer, whereby groups that behave collectively could transfer and utilise social information to a greater degree than less collective groups, facilitating a faster assessment of the actual risk of an environment. However, due to the complex interactions that occur between individuals in a

freely moving group, this design did not allow us to test whether information transfer was the key mechanism that drove the correlation between groups' collectiveness and their rate of habituation. A group-based study also did not allow us to investigate the effects of individual personality traits on the rate at which individuals habituate to novel environments.

The second data chapter used an individual-based design, where the rate of habituation of a focal individual was tested for correlations with their tendency to behave socially, as well as with an aspect of their personality (an individual's boldness). This study allowed us to provide artificial social cues for the focal individual by placing a group of conspecifics within an enclosure in the open area, and investigate how the degree to which an individual acquired this information affected the rate at which it habituated to the novel environment. This study found no significant correlation between an individual's sociability and the degree to which they habituated to the environment.

Although a positive correlation between groups' collectiveness at the start of a trial and their rate of habituation was observed during the first data chapter, the majority of individuals trialed during both of the studies did not show signs of environmental habituation over the course of the trials. This may be due to some limitations in the methods used in these two studies. For example, in both studies, no food patches were present within the open areas. This essentially meant that there was no potential benefit associated with exploration of the open area, which may have deterred individuals from exploring the open area more once they had habituated to the environment. Rather, in these studies, it is likely that the most optimal use of an individual's energy was to spend more time in the refuges once they had established that the environment was bare of resources, rather than spend more energy exploring the bare environment (Lima 1984). As our measures of habituation were all based on the time that an individual spent in the open area over the course of a trial (whereby groups were considered to have habituated if they increased the time spent within the open area as the trials progressed), these may have not been suitable indicators of an individual's degree of habituation in these environments.

These methods can be refined in future studies by providing rewards for exploring the risky open area, such as food patches distributed around the open area, or food that is released once individuals cross the open area, similar to the method used in McDonald *et al.* (2016). This would more closely replicate the natural environment of sticklebacks, where there are benefits to exploration, in terms of a higher probability to come across a food patch, as well as costs, in terms of a higher risk of predation in open areas.

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